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The Effects of Different Feed Supplements on Performance Parameters, Egg Measurements, and Eggshell Integrity in Older White Leghorn Laying Hens

Josephine Foley
University of Nebraska-Lincoln, jfoley@unl.edu

THE EFFECTS OF DIFFERENT FEED SUPPLEMENTS ON PERFORMANCE PARAMETERS, EGG MEASUREMENTS AND EGGSHELL INTEGRITY IN OLDER WHITE LEGHORN LAYING HENS

by

Josephine N. Foley

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Josephine N. Foley, M.S.

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Advisor: Sheila E. Purdum

Two studies were conducted to evaluate the efficacy of multiple feed additives on the performance of older White Leghorn hens. Study 1 examined the effects of 2 vitamin/mineral supplements and 1 medium chain fatty acid. Study 2 examined the effects of a novel butyric acid product, a tributyrin ester. Both studies followed a completely randomized design with repeated measures and data were analyzed using the Glimmix procedure in SAS version 9.4 for Windows.

The first study took place November 2015 to April 2016. Trt 1 was a control diet, Trt 2 was supplemented with a Ca, Fe, Mn, Cu, Zn mixture, Trt 3 with a medium chain fatty acid, Trt 4 and Trt 5 with a Ca, Vitamin D_3, Mn, Zn mixture. A total of 270 Bovan White Leghorn hens were housed in 45 cages. Measurements included: egg production, feed intake, mortality, egg weights, eggshell breaking strength, eggshell percent, and Haugh Unit. Significant differences were seen for feed intake ($p<0.0007$) and egg weight ($p<0.0012$). No significant differences were found for egg production ($p<0.14$), eggshell breaking strength ($p<0.1238$), eggshell percent ($p<0.7974$), or Haugh Unit ($p<0.6240$).

The second study ran from June to September 2016. Trt 1 was fed a control diet and Trt 2 was supplemented with a tributyrin ester at a rate of 0.055%. A total of 144 Bovan White

Leghorn hens were housed in 24 cages. Measurements included: mortality, feed intake, egg production, egg weight, egg mass, eggshell percent, eggshell breaking strength, instance of shell deformities, calcium and phosphorus digestibility. No differences were found for feed intake ($p<0.9027$), egg production ($p<0.2857$), egg weight ($p<0.1346$), egg mass ($p<0.2618$), eggshell percent ($p<0.8470$), eggshell breaking strength ($p<0.0876$), instance of shell-less eggs ($p<0.2973$), calcium digestibility ($p<0.9740$) or phosphorus digestibility ($p<0.2834$).

These results show that some improvement can be seen when hens are supplemented with various minerals and fatty acids, with continued research investigating combinations and inclusion rates.

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Chapter 1: Literature Review

Introduction

In the last 40 years, egg consumption has tripled and is continuing to rise (Zaheer, 2015, Windhorst, 2011), which puts higher demands on laying hens. This becomes more challenging over time, as producers experience an increase in eggshell breakage and a decrease in overall production as the hen ages. Because the hen mobilizes calcium from her bones, osteoporosis (cage layer fatigue) becomes a concern in older hens. Osteoporosis may result in a spike in both mortality and morbidity, resulting in economic losses for farmers (Webster, 2004). The hens' digestive tract is also affected. Weakening mucosal cells in the intestinal wall and shortening villi lead to a decrease in feed efficiency linked to decreased nutrient absorption and decreased calcium and phosphorus available in circulation. In addition to these digestive changes, the egg also changes. Egg size increases as the hens' age, but shell percent does not increase, resulting in thinner and weaker eggshells.

Eggshell quality is the most significant loss for the egg industry (Swiatkiewicz et al., 2015). In fact, cracked eggs may account for up to 10% of laid eggs in young hens and up to/exceeding 20% in older hens (Pavlovski et al., 2012, Nys et al., 2001, Roland, 1988). While this leaves huge economic implications for farms, this also increases risk for the consumer. The eggshell is the first line of defense against pathogenic bacteria, particularly Salmonella. Therefore, improving eggshell integrity is crucial to protect consumers.

To combat these challenges, researchers have spent decades improving genetics, improving management, and improving nutrition. Geneticists have worked to breed a more efficient hen. Engineers research various ways to decrease egg losses, from processing equipment to handling and husbandry practices. However, nutrition is the main area of development to address the challenges facing laying hens and the egg industry. When looking into supplements, there are two overarching goals: to control pathogens and to enhance gut health and overall performance. The resulting increase in feed efficiency increases egg yields and improves shell quality.

These goals may be achieved through macro/micro nutrient supplements, probiotics, prebiotics, essential oils and organic acids (Figure 1.1). Increasing calcium content in the diet can help with eggshell strength, but alone it is not sufficient to combat increasing eggshell weakness. Too much calcium may lead to a decrease in feed consumption, which can cause nutrient deficiencies of other nutrients (Pavlovski et al., 2012). Eggshell formation is also a balance of multiple nutrients, namely calcium and phosphorus and vitamin D and manganese (Sengor et al., 2007). Therefore, supplements look at combinations of nutrients that together improve shell integrity (calcium, phosphorus, vitamin D_3, zinc, manganese, copper, etc.).

Macro nutrients

Calcium, phosphorus, and vitamin D_3 are the 3 macro nutrients of most interest in the laying hen diet. Calcium, one of the most abundant minerals in livestock, is best known for its role in bone, teeth, and shell mineralization. In fact, calcium is one of the two most abundant minerals required in poultry, the second being phosphorus. Thorough research has been done looking at level, particle size, and source of calcium. The Eggshell consists of approx. 95% calcium carbonate, so an optimal supply of usable calcium is one of the most important nutritional factors in highly productive egg laying hens (Swiatkiewicz et al., 2015). Previous studies suggest that the NRC listed value for calcium is adequate for optimal shell formation and that further increases in calcium above 3.6% - 3.9% has no positive influence on eggshell quality

(Bar and Vax, 2002, Keshavarz, 2003, Valkonen et al., 2010, Pastore et al., 2012, Swiatkiewicz et al., 2015). A study done in 2010 disagreed, finding that increasing calcium levels to 4% resulted in increased egg production, egg mass, feed consumption, shell weight, and calcium concentration in the egg shell (El Maksoud, 2010). Other studies suggest particle size can positively influence eggshell quality (Pavlovski et al., 2003, Koreleski and Swiatkiewicz, 2004, Lichovnikova, 2007). For example, coarse limestone offers prolonged retention in the gizzard and, therefore, a steady supply of calcium for many hours. In contrast, dietary calcium, like small particle limestone or dicalcium phosphate, quickly passes through the gizzard and the gastrointestinal tract as a short term calcium source. This is of particular interest overnight, when the shell is being added and the hen is not eating. A study by Swiatkiewicz in 2015 reported that increasing calcium to 4.2% had no effect on eggshell parameters, but substituting a portion of the fine particle limestone with large particle limestone positively impacted eggshell percentage, thickness, density and breaking strength in older laying hens (69 weeks of age) in an ISA brown hen flock. Another study found that calcium supplementation above the NRC recommended level resulted in increased plasma and eggshell calcium and eggshell phosphorus (Bolukbasi et al., 2005).

Another influential macro nutrient is phosphorus, which is essential for numerous body functions, namely bone formation and egg development. The 1994 NRC listed value for available phosphorus (AP) for hens is 0.45%, but studies have shown that supplementing and increasing AP can improve egg production, especially in older hens when osteoporosis is of more concern. A study by Snow in 2004 found that increasing AP to 0.45% showed an increase in body weight, egg mass and feed intake in first cycle hens. Snow also found increased egg production, egg mass, and feed efficiency in second cycle hens fed lower levels of AP as compared to those fed 0.45% AP, showing that AP may be needed in higher quantities in older hens. Another study performed in 2009 found improved breaking strength in groups supplemented with calcium and phosphorus, versus a basal control group (Sharma et al., 2009).

Vitamin D_3 is also crucial for bone formation and low vitamin D_3 levels may result in osteoporosis (Lips et al., 2001). It is believed that vitamin D_3 plays a role in optimal intestinal function, and it has been established that a vitamin D_3 dependent Ca binding protein is key in transporting calcium across the intestinal membrane (Bolukbasi et al., 2005, Keshavarz, 2003). Therefore, vitamin D_3 supplementation may increase calcium deposition and calcium circulation. Through this increase in calcium transport across the intestinal membrane, calcium available to the hen and calcium in the eggshell may be positively impacted. In 2005, a study reported that vitamin D_3 supplementation increased calcium concentration in plasma and eggshell, and increased phosphorus concentration in plasma. (Bolukbasi et al., 2005). This study also noted that adverse effects of calcium supplementation were decreased by adding vitamin D_3 to the diet. A separate study found that increased levels of calcium concentration in the blood plasma and eggshell and improved egg production in hens fed a vitamin D_3 supplement of 2500 IU/kg or 4000 IU/kg (El Maksoud et al., 2010). This study also found the highest level of egg production, egg weight, egg mass, feed consumption, shell weight and calcium concentration in the eggshell in hens fed a vitamin D_3 supplement in conjunction with a calcium supplement. A study in 2004 found improved bone strength in hens fed higher levels of vitamin D_3 in the diet, which agrees with the findings of studies in 2002 (Mattila et al., 2004, McCormack et al., 2002, Whitehead, 2002).

Micro Nutrients

Micro minerals are believed to influence eggshell integrity by acting as key enzymes involved in the membrane and shell formation process or by interacting with the crystal structures in the eggshell (Mabe et al., 2003). For example, zinc and manganese are cofactors of metaloenzymes responsible for carbonate and mucopolysaccharides synthesis (Swiatkiewicz and Koreleski, 2008). Manganese activates transferases that are involved in the formation of mucopolysaccharides which are key components of keratin and dermatan proteoglycans. These proteoglycans may influence the structure and texture of the eggshell and are present in the eggshell matrix (Arias et al., 1993, Nys, 2001). Zinc is important for the carbonic anhydrase enzyme, which is a key component for circulating carbonate ions during the eggshell formation process (Mabe et al., 2003). Zinc, manganese, and copper may affect eggshell integrity by influencing calcite crystal formation and the crystallographic structure of the eggshell (Mabe et al., 2003). This would influence the mechanical properties of the eggshell. Some studies suggest that organic sources of these micro minerals have a higher bioavailability (Li et al., 2005, Yan and Waldroup, 2006, Ao et al., 2006). This may be linked to different mechanisms of absorption for inorganic versus organic mineral complexes and protection from binding to dietary elements and forming indigestible complexes (Swiatkiewicz et al., 2001). Some studies found that organic sources of micro minerals resulted in higher eggshell weight and thickness late in the lay cycle (Klecker et al., 2002, Swiatkiewicz, 2008), but other studies found no difference when compared to inorganic mineral sources (Mabe, 2003, Lim et al., 2003).

Multiple studies have found that supplementing with trace amounts of zinc in older laying hens improves eggshell thickness and strength (Zamani et al., 2005, Guo et al., 2002), One study in 2011 found increased feed intake and egg production in zinc supplemented hens

(Idowu et al., 2011). Zinc may even decrease barn ammonia levels by reducing decomposition of uric acid and increasing manure total nitrogen, but that high levels of zinc may depress hen performance (Kim and Patterson, 2004).

In 2001, a study by Inal reported that 25 mg manganese/kg was sufficient for optimal egg production and weight, but for optimal shell quality the amount is much higher. Therefore, supplementation of manganese may promote improved eggshell integrity. In 2005, Zamani found that supplementing zinc and manganese in combination in older White Leghorn hens significantly increased shell percent, breaking strength, elastic modulus, stiffness, fracture toughness, and calcium content of the eggshell. A study by Mabe in 2003 strengthened this view, finding improved eggshell breaking strength and resistance to fracture in older ISA Brown hens supplemented with zinc, copper, and manganese, and that these positive results were observed regardless of mineral source (organic vs inorganic). Another study noted that egg production and eggshell strength improved in Cu-methionine chelate supplemented hens (Lim et al., 2003). A different study by Paik in 2009 reported improved egg weight, Haugh Unit, and iron content of the yolk in hens supplemented with iron. Egg production was also improved in broiler breeders supplemented with inorganic and complexed iron (Bess et al., 2012).

Organic Acids – Short Chain and Medium Chain Fatty Acids

Organic Acids are natural substances present in biological liquids, tissues, and the gastrointestinal tract of animals or plants (Ricke, 2003, Guilloteau et al., 2010). They are produced in the end of the gastrointestinal tract in humans and food animals due to fermentation of carbohydrates (Ricke, 2003). Short chain fatty acids are a group of simple monocarboxylic molecules that contain from 1 to 7 carbon atoms in either straight or branched chains. (Guilloteau et al., 2010). Medium chain fatty acids consist of 6-10 carbon atoms (Van Immerseel et al., 2004). Short chain fatty acids are absorbed throughout the entire gastrointestinal tract, most notably in the small and large intestines through similar mechanisms. In the basolateral membrane of the intestine, a carrier-mediated HCO3- gradient-dependent anion-butyrate exchange system is believed to be the mode of butyrate uptake.

Theories of the antimicrobial activity of organic acids date back to 1906 (Winslow and Lockeridge, 1906). It is believed that animal performance is improved due to the increased nutrient digestibility, stimulation of digestive enzymes, modification of intestinal microflora and improvements in intestinal epithelial integrity (Guilloteau et al., 2010). There is evidence that fatty acids may influence the availability of Ca and other minerals in circulation, which may improve eggshell quality. For instance, fatty acids reduce intestinal pH, which may alter the activity of certain digestive enzymes, leading to an increase in available minerals. Organic acids also stimulate the villi, increasing villi height and cells lining the gastrointestinal tract. Many studies have shown that the addition of various organic acids, including short chain and medium chain fatty acids, has a beneficial impact on laying performance and eggshell quality (Park et al., 2002, Yesilbag and Colpan, 2006, Sengor et al., 2007, Soltan, 2008).

In 2010, a study by Swiatkiewicz found that a mixture of short chain and medium chain fatty acids had a significant benefit to eggshell quality in Bovans Brown hens (46 + weeks). Medium chain fatty acids had the most positive effect on eggshell percent, shell density, and shell strength at 46+ weeks. The researcher credited these results to increased availability of Calcium and Phosphorus due to the decrease in intestinal pH. Medium chain fatty acids improved eggshell strength and egg weight in Lohmann Brown hens aged 30-32 weeks (Klementaviciute et al., 2016). Medium chain fatty acids improved egg production, eggshell strength, Haugh unit, increased serum calcium concentration, increased fecal Lactobacillus and decreased fecal E. coli in Hy-line brown laying hens aged 25 weeks (Lee, 2014). Studies have suggested that medium chain fatty acids have the greatest bacterial effect on Salmonella (Van Immerseel et al., 2006), and they have this effect at lower concentrations than a similar effect using short chain fatty acids (Van Immerseel et al., 2003).

A 2020 survey of broiler nutrition professionals found that butyric acid was the second most widely used supplement to replace antibiotics in broiler feed (Mavromichalis, 2020). Naturally found in the gastrointestinal tract, milk, sweat and feces of most mammals, butyric acid is available as the sodium, potassium, Magnesium or calcium salt. (Guilloteau et al., 2010). Salts are more desirable due to the odorless and more stable characteristics, making them more palatable and easier to handle when manufacturing. Butyrate is a natural component of cellular metabolism in all tissues and has been shown to act as a growth promoter in small doses (Guilloteau et al., 2010). Butyrate is known to decrease bacterial virulence by directly damaging pathogen cells and by changing the pathogens environment within the host. With a pKa value of approx. 4.8, this weak acid is shown to have bacteriostatic and bactericidal properties depending on the pathogen and the intestinal environment (Ricke, 2003). Within the gastrointestinal tract, butyrate is known to have numerous positive effects on feed efficiency, growth and digestibility by modulation of cell proliferation, differentiation and function in the gastrointestinal tract. This is especially true for mucosal epithelial cells and on defense systems, including the immune system, barrier function, and antimicrobial potency in both healthy and sick animals (Guilloteau et al., 2010).

Organic acids enhance gut health in multiple ways, including lowered pH, slowed gastric emptying, and increased digestive enzyme secretions that increase the metabolism of nutrients.

Lowering the overall pH of the gut improves the solubility of feed ingredients, thus making them more digestible. Easier to digest ingredients means improved absorption and therefore, increased nutrients in circulation and available to the animal.

Significantly improved weight gain and feed efficiency has been found in broilers supplemented with butyric acid (Panda et al, 2009, Adil et al 2010, 2011). A 2011 study reported that supplementing with short chain fatty acids improved metabolizable energy and overall nutrient digestibility (Ghazala et al, 2011). Two studies in 2006 and 2007 found similar results with improved apparent ileal digestibility of dry matter and crude protein in a broiler finisher diet that included short chain fatty acids (Hernandez et al, 2006, Garcia et al, 2007).

Soybean meal is known to have poor digestibility rates in poultry due to birds lack of endogenous alpha-(1, 6)-galactosidase enzyme in the small intestine. This leaves them unable to digest the galacto-oligosaccharides in the SBM (Lee et al, 2014). In 2005, a study reported that by decreasing the pH in the crop, the activity of alpha-galactosidase was enhanced (Ao, 2005). Afsharmanesh & Porreza found that the decrease in pH of the chyme enhanced digestibility of protein by increasing pepsin activity in another study done in 2005 with broilers.

Enzyme secretion activity has been found to increase when organic acids are present. In 2010, a study reported that pancreatic secretion activity was enhanced with the supplementation of organic acids (Adil et al, 2010). The increase in trypsinogen, chymotrypsinogen A, chymotripsinogen B, procarboxy peptidase A and procarboxy peptidase B led to improved digestion of proteins. In 2016, Sobczak found increased activity of enzymes including beta-glucosidase, alpha and beta-galactosidase, beta-glucuronidase and beta-sylosidase.

Organic acids can slow the passage of feed. It is believed that organic acids may play a role in slowing down the rate of passage through the GIT, delayed gastric emptying means better absorption and less wet droppings (Van Der Sluid, 2002). In 2018, a broiler study by Moquet found statistically significant increased small intestine retention time. The researcher believes this was caused by neuroendocrine mechanisms that responded to butyrate. Another study reported greater retention of dry matter, crude protein, and gross energy in hens supplemented with sodium butyrate (Chou et al., 2014).

Several studies have shown that organic acid supplementation improves the digestibility of key minerals and improve utilization of phytate phosphorus (Park et al, 2009). Acidifying the environment improves phytase activity. Increasing total phosphorus solubility may result in prolonged time in the small intestine (Han et al, 1998). Increasing acidity is also known to increase solubility of key minerals, therefore calcium effectiveness may be increased (Khan and Iqbal, 2015). A broiler study found that increased butyrate in the large intestine and ceca increased apparent ileal digestibility of methionine and histidine (Moquet et al., 2018). This agreed with findings from a study in 2017, a broiler study that found increased ileal digestibility and energy. The supplemented birds also showed increased body weight (Liu et al., 2017).

In 2010, researcher Adil found increased serum calcium and phosphorus concentrations in birds receiving an organic acid. In 2012, Swiatkiewicz and Arczewska-Wlosek reported increased calcium content in the bones of hens fed organic acids. This study also found greater strength in the tibia and femur, which suggests a lower release of calcium from the bones due to improved absorption of calcium in the gastrointestinal tract. In 2016, Sobczak also found a higher calcium content in the bones of birds receiving an organic acid.

Organic acids are involved in the development of healthy gut wall. Acids provide energy to epithelial cells (Gadde et al., 2016, Guilloteau et al., 2010). Butyric acid is the primary energy source of epithelial cells in poultry, is necessary for the proper development of gut associated lymphoid tissue, and is considered an important growth modulator of intestinal microflora (Leeson et al, 2005). Acids stimulate villi growth. increase epithelial cell propagation, and decrease apoptosis of normal enterocytes. This increases the surface area of the small intestine, which increases overall nutrient absorption. In 2016, Sobczak found improved ileal parameters including thickness of glandular layer, villus height and crypt depth in supplemented birds. In 2010, Adil found significantly increased intestine length in the organic acid supplemented group.

Short chain fatty acids are shown to enhance healthy tissue turnover and maintenance by stimulating production of normal crypt cells, with butyrate showing the strongest effect (Salminen, 1998). Butyrate was found to facilitate DNA repair enzymes (Langhout and Sus, 2005) and increased villus height, crypt depth and surface area in the colon of rats supplemented with butyric acid (Frankel et al, 1994). In 2017, Liu found increased villus height in the duodenum and jejunum of broiler chickens in 2 different broiler trials testing organic acids. In one, broilers were challenged with Salmonella. The other was a grow out study using encapsulated sodium butyrate that found improved villus height to crypt depth ratio in the ileum of broilers.

Broilers fed butyrate at 0.2, 0.4, or 0.6% rates showed improved villus length and crypt depth in the duodenum (Leeson et al, 2005, Panda et al, 2009). Highest villus height in birds fed short chain fatty acids in the duodenum, jejunum and ileum. Muscularis thickness was decreased in these birds as well (Adil et al, 2010). Increased villus height may also play a role in pathogen control as the intestinal epithelium acts as a natural barrier against pathogens and toxic substances (Khan, 2015). In 2009, a study reported that fat-coated organic acid supplementation increased nitrogen retention in birds (Smulikowska et al, 2009). An increased nitrogen retention may account for increased epithelial cell proliferation in the gastrointestinal tract.

Sources of Butyric Acid

Butyric acid is available in multiple forms. Fat-coated or encapsulated is particularly useful as encapsulation delays butyric acid digestion, allowing it to make it farther in the gastrointestinal tract before being broken down. This targeted release allows for the benefits of butyric acid to be seen in the small intestine. A 2007 study reported that fat-coating of the acid prevented dissociation in the stomach and allowed the acid to reach distal parts of the intestine (Hu and Guo, 2007). This addressed the acids bioavailability in farther parts of the gastrointestinal tract to effectively improve intestinal microflora and mucosal morphology in chickens.

Unprotected butyrate is active in the crop, proventriculus, and gizzard. Tributyrin is active in the small intestine (Moquet et al, 2016). The target in many poultry studies is the small intestine as butyric acid earlier in the GIT may promote an anorexic response (Moquet et al., 2018).

In recent years, a new form of butyric acid has been formed by the Perstorp company[1]. They developed a tributyrin ester, or a glycerol ester consisting of 3 butyrate molecules attached to a glycerol backbone.

Organic Acids and Egg Characteristics

Organic acid supplementation has been repeatedly found to have positive effects on egg production in laying hens. In 2006, a supplemented group of hens aged 24-28 weeks showed greatly improved egg production that stayed elevated until the end of the trial at 36-38 weeks

[1] Perstorp Holding AB Neptunigatan 1, 211 20 Malmo, Sweden

when compared to the control (Yesilbag and Colpan, 2006). Two other studies reported similar results. Hens aged 70 weeks supplemented with organic acids showed significantly increased egg production compared to the control fed a basal diet (Soltan, 2008, Rahman et al, 2008). These trials also found improved nutrient utilization and feed conversion and improved hen body weight. In 2008, Soltan found no differences in egg weight and no effect on feed intake. Rahman also found no effect on feed intake, egg weight or hen weight, but improved feed conversion and significantly improved egg production in a study conducted in 2008. These findings disagreed with two studies by Chou and Wiltenburg which found no effect on egg production (Chou et al., 2014, Wiltenburg and Lee, 2005).

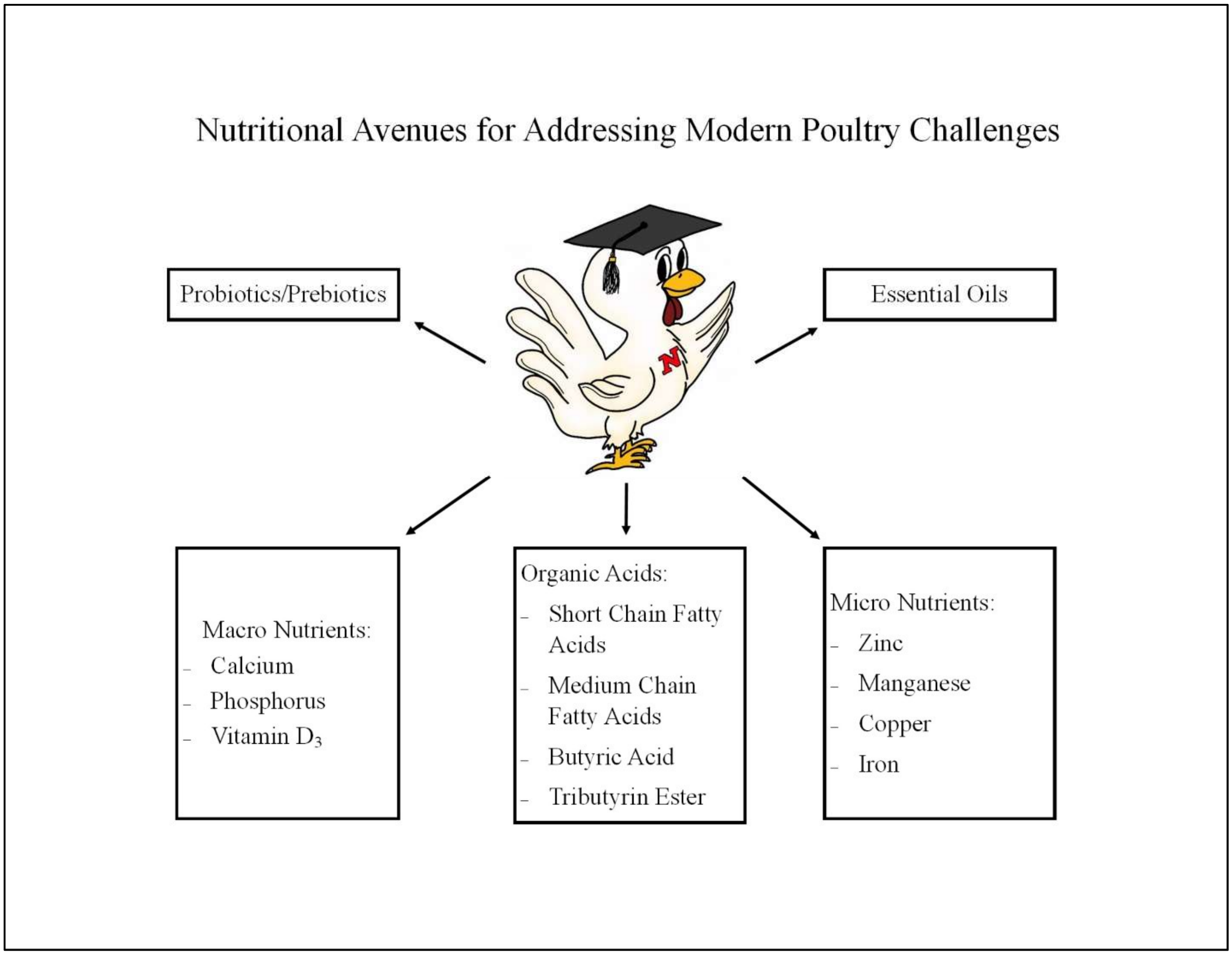
In 2005, Langhout and Sus reported increased egg weight when the diet included an organic acid. Another study found that hens supplemented with an encapsulated organic acid showed significant improvements in egg production, feed conversion and feed intake compared to the control (Youssef et al, 2013). Rahman disagreed with this when they found no effect of organic acid supplementation on feed intake in a trial done in 2008. A study in 2002 reported that eggs from hens supplemented with an organic acid mixture showed markedly improved eggshell strength (Park et al, 2002). Two trials conducted with old hens agreed with these findings with an increased eggshell thickness, increased eggshell calcium content and decrease in number of broken eggshell occurrences (Soltan, 2008, Rahman et al., 2008). It is suggested that this improvement in eggshell strength and eggshell thickness may be due to the increased mineral and protein absorption. In 2016, Sobczak reported increased eggshell thickness and percent eggshell, but no increase in breaking strength.

A study in 2007 found that breeder hens supplemented with a short chain fatty acid premix at 66-weeks of age showed markedly improved eggshell strength versus the control. The percentage of dirty, cracked or misshapen eggs and hatchability was also significantly improved in the supplemented group (Sengor et al., 2007). In 2014, Chou found decreased incidence of broken eggs and increased eggshell strength.

Conclusion

With egg consumption increasing globally, eggshell breakage is a top concern for egg producers. Eggshell breakage accounts for a large economic loss to the producer and an increased bacterial risk to the consumer. This places many demands on the hen, with challenges increasing as the hen ages. Researchers look to many avenues to combat these challenges, nutrition being one huge area of development. Many products are being studied and macro or micro nutrients and fatty acids show promising results in trials. A combination of good management and husbandry processes with appropriate combinations of various supplements may be key in maximizing bird performance (Gadde et al., 2016). While the performance of organic acids lacks consistency, the variability can be accounted for with different sources and inclusion rates of the organic acids and the unique buffering capacity of other dietary ingredients (Gadde et al., 2016).

Figure 1.1: Nutritional Avenues for Addressing Modern Poultry Challenge[2]



[2] Created manually by Josephine Foley

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Effective supplements for maintaining egg shell quality late in the laying hen cycle.

By: J. Foley*[†], K.J. Hanford[†], S. Purdum[†]
[†] University of Nebraska – Lincoln
*Student Presenter

ABSTRACT: In recent years, the poultry industry has experienced an onslaught of issues associated with H5N2, also known as Avian Influenza. Due to the outbreak of Avian Influenza, many laying hen facilities have been keeping hens in production much longer than their normal production period. As the hen ages, shell quality deteriorates leading to increased numbers of broken eggs which take a toll on the industry economically. For this reason, it is important to test products that may improve shell quality and bone health. This study sought to investigate the effectiveness of 3 different feed additives on shell strength and bone health in older white Leghorn hens. A total of 270 Bovan White laying hens were housed in 45 cages for a total of 24 weeks (6 months) from 58 weeks to 82 weeks of age. Each cage was assigned to one of five treatment groups with 9 replicate cages in a randomized complete block design. Group 1 was assigned as the control group, group 2 was supplemented with LayerMax Shell Add Pack, Group 3 was supplemented with EXPMCFA, Group 4 was supplemented with Fortishell for 24 weeks, and Group 5 was supplemented with Fortishell for 10 weeks, fed the control diet for 6 weeks, and fed Fortishell for another 8 weeks. Egg production, feed consumption, and mortality were recorded daily. Egg weights were measured and recorded biweekly. Eggshell strength and shell percent were measured biweekly, and Haugh Unit was measured monthly. A higher feed intake was calculated at a gram/hen/day rate in Groups 4 and 5 ($p<0.0007$). The control group showed a markedly lower feed intake overall. A larger egg weight was noted for Groups 2 and 5 ($p<0.0012$), and the lowest average weight was measured in the control group. Finally, a higher egg shell breaking strength was found in Group 5 ($p<0.12$). Based on these results, this study found that certain supplements did successfully improve egg shell integrity in older laying hens compared to a control.

KEY WORDS: Laying hen, feed additives, egg production.

Chapter 2: Effective supplements for maintaining egg shell quality late in the laying hen cycle.

Introduction

As egg consumption increases, pressure on laying hens also increases. However, the hen faces more challenges as she ages. Egg size increases as the hen ages, but eggshell amount stays approximately the same. This results in thinner, weaker shells (Arpasova et al., 2010). Instance of osteoporosis also increases as the hen ages. Also known as cage layer fatigue, this results in an increase in morbidity and mortality in hen houses. Cracked eggshells and increased mortality are a large area of economic loss for farmers (Webster, 2004). For this reason, researchers have spent decades looking for ways to combat these challenges. From genetics to engineering and management, nutrition is the main area of development to address challenges to the laying hen. Many supplements have been looked into containing various nutrients, such as calcium, phosphorus, Vitamin D_3 and other minerals.

The laying hen requires an extraordinary amount of calcium due to the high content of calcium in egg shells, therefore, limestone (calcium carbonate) is one of the most abundant dietary ingredients used with egg laying birds. The eggshell consists of approximately 95% calcium carbonate, so an optimal supply of available calcium is considered one of the most important nutrients in highly productive laying hens (Swiatkiewicz et al., 2015). Some studies have suggested that the NRC listed calcium value is adequate and that increasing the calcium value to over 3.6-3.9% has no positive influence on eggshell quality (Bar et al., 2002, Keshavarz, 2003, Valkonen et al., 2010, Pastore et al., 2012, Swiatkiewicz et al., 2015). However, many studies have disagreed, finding positive results from increasing calcium levels alone or in addition to other supplements.

Considered the second most important mineral, phosphorus is essential for numerous body functions, namely bone formation and egg development. The NRC recommends a dietary level of 0.25% available phosphorus, but studies have shown that increasing available phosphorus can improve production, especially in older laying hens when osteoporosis is a concern. In 2004, a study found that increasing available phosphorus to 0.45% showed an increase in body weight, egg mass and feed intake in first cycle hens (Snow et al., 2004). This study also found increased egg production, egg mass, and feed efficiency in molted hens when compared to hens fed lower quantities, indicating that available phosphorus may be needed in higher amounts in older laying hens (Snow et al., 2004). A study in 2009 found improved breaking strength in groups of hens supplemented with both calcium and phosphorus versus a basal control group (Sharma et al., 2009).

Vitamin D_3 is important for bone formation, low levels of Vitamin D_3 may result in osteoporosis. Nutritionists believe that Vitamin D_3 plays an important role in maintaining optimal intestinal function and that a Vitamin D_3 dependent calcium binding protein is key in transporting calcium across the intestinal membrane (Keshavarz, 2003, Bolukbasi et al., 2005). Therefore, Vitamin D_3 supplementation may increase calcium deposition in the bone, calcium in the plasma, and calcium in the eggshell through this increased calcium transport process in the intestinal membrane.

In addition to calcium and phosphorus, microminerals may influence eggshell integrity as well. Some minerals are involved as key enzymes in the membrane and shell formation process or they interact with the crystal structures in eggshell formation (Mabe et al., 2003). Zinc and manganese are cofactors of metaloenzymes responsible for carbonate and mucopolysaccharide synthesis (Swiatkiewicz and Koreleski, 2008). Mucopolysaccharides are components of proteoglycans, which are important parts of bone structure and in the eggshell matrix (Mabe et al., 2003). Manganese specifically activates transferases that are involved in the formation of mucopolysaccharides found in keratin and dermatan proteoglycans. These proteoglycans are found in the eggshell matrix and influence the structure and texture of the eggshells, potentially playing a role in eggshell integrity (Arias et al., 1993, Nys, 2001). Zinc is also known to play an important role with carbonic anhydrase enzyme, a critical component for circulating carbonate ions during the eggshell formation process. In addition, zinc, copper and manganese play roles in influencing calcite crystal formation and the crystallographic structure of the eggshell, which may also influence the mechanical properties of the eggshell (Mabe et al., 2003).

In addition to minerals, nutritionists have been exploring other feed supplements to improve shell quality. Short and medium chain fatty acids are supplements being considered. Evidence has shown that fatty acids may influence the availability of calcium and other key minerals in circulation (Adil et al., 2010). Improved digestion and absorption could positively impact performance parameters. Fatty acids are known to decrease intestinal PH (Adil et al., 2011), which could impact the activity of key digestive enzymes, leading to an increase in availability of minerals to the hen. Fatty acids are also known to stimulate intestinal villi, both by increasing villi height and impacting cells lining the gastrointestinal tract, which would improve overall gastro function (Adil et al., 2010).

Several studies have found that the addition of short chain and medium chain fatty acids has a beneficial impact on laying performance (Park et al., 2002, Yesilbag and Colpan, 2006, Sengor et al., 2007, Soltan et al., 2008). A study in 2010 found that supplementing a mixture of both short chain and medium chain fatty acids showed a significant improvement in eggshell quality in older Bovan Brown laying hens (Swiatkiewicz et al., 2010). The most positive impact on eggshell percent, eggshell density, and eggshell strength in older hens was the medium chain fatty acid group. These effects are believed to be linked to an increased availability of calcium and phosphorus in circulation due to the decrease in intestinal pH and a stimulating effect on the intestinal villi (Swiatkiewicz et al., 2010). These results are in agreement with studies done in 2007 that found an improvement in body weight gain, feed conversion ratio, villi height, crypt depth, and apparent ileal digestibility in a fatty acid supplemented group (Garcia et al., 2007, Senkoylu et al., 2007).

This study focused on investigating multiple supplements containing different combinations of minerals and medium chain fatty acids on performance parameters in older laying hens.

MATERIALS AND METHODS

Birds and Housing

A flock of 270 Bovan White Leghorn hens aged 58 weeks were used for this 24-week trial (ISA North America, Ontario, Canada), from 58 to 82 weeks of age. The trial ran from November 2015 to April 2016. Hens were housed in 45 cages in a Big Dutchman manure belt battery cage system, with 3 tiers of 8 cages per side and 6 hens per cage. Cages measured approx.. 18 inches tall in the front, 15.75 inches tall in the back, 24 inches wide, and 20.25 inches deep, providing hens with approx.. 81 inches ^{2}/hen. Water was available ad libitum via nipple drinker at the back of the cage and hens were provided with 110 grams/day of feed. The photoperiod consisted of 16 hours of light and 8 hours of dark per day, provided by an automated lighting system. Each cage was randomly assigned to 1 of 5 treatment diets with 9 replicates per treatment using a completely randomized design. The conditions of the trial were approved by the Institutional Animal Care and Use Committee at the University of Nebraska – Lincoln and the trial was held in Poultry Building F on East Campus.

Diets

The independent variable of this trial was diet and consisted of 5 treatments. Treatment 1, the control diet, consisted of a typical corn-soybean basal diet that follows the NRC laying hen nutrient recommendations of 1994. The complete control diet composition can be seen in Table 2.1. Treatment 2 consisted of the control feed with LayerMax Shell Add Pack, a supplement consisting of 16.7% Ca, 5,500 ppm Fe, 5.92% Mn, 405 ppm Cu, and 19,200 ppm Zn. Treatment 3 contained the control feed with an experimental combination of medium chain fatty acids (proprietary mixture including caproic acid, caprylic acid, capric acid, and lauric acid). Treatment 4 consisted of the control feed plus Fortishell containing 23.5% Ca, 500,000 ICU/lb Vitamin D_3, 6.5% Mn, and 1.9% Zn known as Fortishell. Finally, treatment 5 was made up of the control feed with the Fortishell supplement fed for 10 weeks, then the control feed alone for 6 weeks, and finished with the control feed with Fortishell fed for the last 8 weeks. All supplements were provided by PMI, Purina (Land O' Lakes, Minnesota, USA).

Measurements

Egg production, feed consumption and mortality were recorded daily. Egg weight, shell breaking strength, shell percent, and calculated feed conversion was recorded biweekly. Haugh Unit was recorded monthly.

Egg production was recorded on a daily basis and average weekly egg production was calculated by dividing the number of eggs collected by the number of hen days of production.

Eggs were collected once every other week to measure egg weight, shell breaking strength and shell percent. Egg weight was measured by placing the whole egg on a tared scale. The egg was then cracked and the eggshell was weighed after all components were scraped out. Egg mass was calculated by multiplying percent egg production by egg weights. Eggshell breaking strength was analyzed using a texture analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY). The force in Newtons necessary to crack the eggshell was graphed using an exponent software (Stable Micro Systems LTD., Surrey, UK). Haugh Unit was recorded by measuring the height of the albumen and then using the albumen height and egg weight in the Haugh Unit equation. The equation used was h = albumen height and w = egg weight.

$$HU = 100 * LOG (h - 1.7 * w^{0.37} + 7.6)$$

Feed consumption was calculated by taking the total amount of feed weighed out for a set period minus the amount weighed back at the end of a set period. For this trial, the set period was 7 days. This feed intake was then calculated to average consumption per hen per day by dividing the calculated consumption by the number of hens in each cage and the total number of days in the predetermined time period. Feed conversion was calculated by dividing feed intake by egg mass. Mortality was monitored daily. Total number of mortalities were summed per treatment at the end of the trial.

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS, version 9.4 (SAS Institute Inc., Cary, NC, 2015). All response variables were analyzed using a repeated measures model including the fixed effects of time, treatment and their interaction.

RESULTS AND DISCUSSION

This study looked at comparing a basal laying hen diet to diets with various mineral and medium chain fatty acid supplements. The hypothesis was that the increased mineral amounts provided or the addition of a group of medium chain fatty acids would improve overall hen performance and production parameters, with a special interest in eggshell integrity.

Overall, multiple differences were found between treatments, with all 3 supplements showing promise in improving specific parameters. Results are summarized in Table 2.2. Mortality was unaffected by treatment ($p>0.05$).

This study found a significant diet x time interaction ($p<0.0001$) for egg production (Figure 2.1), during several time periods and a significant time effect ($p<0.001$).Trt 1 had the lowest egg production at the end of the study, weeks 18-25, with Trt 3 showing consistent production from weeks 10 to 25. This is in agreement with multiple studies that found supplementing with medium chain fatty acids improved egg production (Chen et al., 2005, Yesilbag and Colpan, 2006). However, these findings disagreed with the study completed in 2010 that found no differences in egg production (Swiatkiewicz et al., 2010). This improvement in egg production in the medium chain fatty acid group may be hypothesized due to improved nutrient absorption due to improved gut health and villi parameters as older laying hens are known to have deteriorated gastrointestinal health (Sengor et al., 2007).

A higher feed intake was calculated as grams/hen/day in Trt 4 and 5 ($p<0.0007$) (Figure 2.2), the groups supplemented with Fortishell. The control group showed a markedly lower feed intake overall, most notably in weeks 13-25. This agrees with several studies that indicated improved feed intake in hens receiving a mineral supplement. El Maksoud and Castillo et al found increased feed consumption in hens fed increased calcium and Vitamin D_3 (El Maksoud, 2010, Castillo et al., 2004). Multiple studies found increased feed intake in hens supplemented with additional available phosphorus or other minerals (Snow et al., 2004, Keshavarz, 2003, Mabe et al., 2003). However, these findings contradicted studies that found no difference in feed intake with hens supplemented with calcium, zinc and manganese (Swiatkiewicz and Koreleski, 2008, Swiatkiewicz et al., 2010, Swiatkiewicz et al., 2015)

A larger egg weight was noted for Trt 2 and 5 ($p<0.0012$) (Figure 2.3), with an average egg weight of 64.27 grams and 64.57 grams respectively, and the lowest average weight was measured in the control group at 62.1 grams. The control group marked the lowest average egg weight during every time period of this study. This supports findings from multiple studies that found improved egg weights in hens supplemented with minerals (Mattila et al., 2004, Zamani et al., 2005, El Maksoud, 2010). However, these findings are in disagreement with other studies that found no effect on egg weight (Swiatkiewicz et al., 2015, Mabe et al., 2003). There may be a correlation between increased feed intake and a higher egg weight in Trt 5, but not for Trt 2.

There was a diet by time interaction for egg mass ($p<0.001$), with an increasing egg mass in all groups over the course of the study ($p<0.001$). Overall, Trt 4 showed the highest average egg mass at 52.02 and the highest mass in weeks 6, 8, 12, and 14. Trt 1 noted the lowest egg mass at 49.14, and the lowest mass in weeks 16, 20, and 22. This agrees with studies that reported an increase in egg mass in hens fed a Vitamin D3 supplement in conjunction with a calcium supplement (El Maksoud, 2010), but disagrees with studies that reported an increased egg mass in hens fed a diet with increased calcium and phosphorus levels (El Maksoud, 2010, Snow et al., 2004).

Treatment had no significant effect on eggshell percent or Haugh Unit (Figure 2.5 and 2.6). However, there were time effects. All treatment groups showed increased eggshell percent in weeks16, 18, and 22. Trt 4 showed notably lower eggshell percent in weeks 2, 3, and 12, but the highest in week 14. Trt 3 showed the lowest eggshell percent in week8, 20, and 24, but the highest in weeks 2, 10, and 18. Trt 1 showed notably higher Haugh Unit scores in weeks 8, 12, and 24, but the lowest in week 20. Trt 3 showed markedly higher Haugh Unit score in week 16, and Trt 2 a higher score in week 20.

There was no overall diet x Trt effects on eggshell breaking strength ($p<0.12$), but a strong trend indicating improvement with Trt 5 (Fortishell weeks 1-10 and 17-22 with a 6-week break) at 56.33 Newtons compared to the control at 53.47 Newtons and other Trts. Trt 5 had a notably higher breaking strength in weeks 2, 8, 10, 14, 16, 18, 20, and 22. This may be linked to increased feed intake in Trt 5, fed the basal diet including Fortishell for 2 periods during the course of the trial. Although Trt 4 also showed increased feed intake, there was no notable increased shell strength in Trt 4, fed a basal diet with Fortishell fed for the entire feeding period. Trt 2 showed an increased breaking strength in weeks 6 and 12, but a markedly lower breaking strength in week 10. Many studies agree with these results, noting increased eggshell strength in hens supplemented with minerals or fatty acids (Swiatkiewicz et al., 2015, Sharma et al., 2009, Inal et al., 2001, Mabe et al., 2003, Guo et al., 2002, Zamani et al., 2005, Swiatkiewicz and Korecleski, 2008).

CONCLUSION

Decreasing mineral availability, deteriorating gut health, and decreasing nutrient absorption in aging laying hens has been a continual challenge to the egg industry for years. Many key minerals, including calcium, phosphorus, zinc, and manganese, in addition to fatty acids, have shown promise in improving production parameters like egg production, feed intake, egg weight and eggshell strength. However, many studies contradict these results and note no beneficial effect on production parameters in egg laying hens that are supplemented with minerals or fatty acids. This variability is to be anticipated as nutritionists continue to narrow the mechanisms and benefits of minerals and fatty acids. Research should continue to look in to source and inclusion rates or combinations of minerals and fatty acids on laying hen performance.

LITERATURE REVIEW

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Table 2.1. Composition of Basal Control Diet

Ingredient (%)	TRT 1
Ground Corn	52.44
Soybean Meal	21.91
DDGS	10.00
Limestone	10.36
Vegetable Oil	3.42
Dicalcium Phosphate	1.00
Salt	0.38
Vitamin/Trace Mineral Premix*	0.20
Methionine	0.19
Lysine	0.10

Nutrient Analysis (%)	TRT 1
Protein	20.98
Lysine	0.98
Methionine	0.57
Calcium	4.25
Phosphorus	0.53
Sodium	0.19
Kcal/kg	3212

*Note: Vitamin/Trace Mineral Premix contained Phytase activity.

Table 2.2. Results Summary

Parameter	TRT1[3]	TRT2[4]	TRT3[5]	TRT4[6]	TRT5[7]	Diet	Time	Diet xTime	SEM
Egg Production, % hen day	79.15	79.66	82.16	81.93	79.23	0.14	0.001	0.0688	1.2198
Feed Intake, g/hen/day	97.15	100.01	101.77	102.57	102.55	0.0007	0.0001	0.6578	0.9299
Egg wt, grams	62.10	64.27	63.37	63.5	64.57	0.0012	0.001	0.001	0.5805
Egg mass, g/hen/day	49.14	51.15	51.99	52.02	51.10	0.14	0.001	0.001	0.2887
Egg Shell, %	0.1385	0.1389	0.1371	0.1375	0.1381	0.7974	0.001	NS	0.0016
Haugh Unit	84.34	84.51	83.04	83.47	82.98	0.624	0.0001	0.772	0.8823
Egg Shell Breaking Strength, N	53.47	54.51	55.48	54.18	56.33	0.1238	NS	NS	1.0693

[3] Trt 1 - Control
[4] Trt 2 – LayerMax Shell Add Pack
[5] Trt 3 - EXPMCFA
[6] Trt 4 – Fortishell (6 months)
[7] Trt 5 – Fortishell (10 and 8 weeks)

Figure 2.1. Egg Production
Diet: NS; Time: P<0.001; Diet x Time: P<0.006

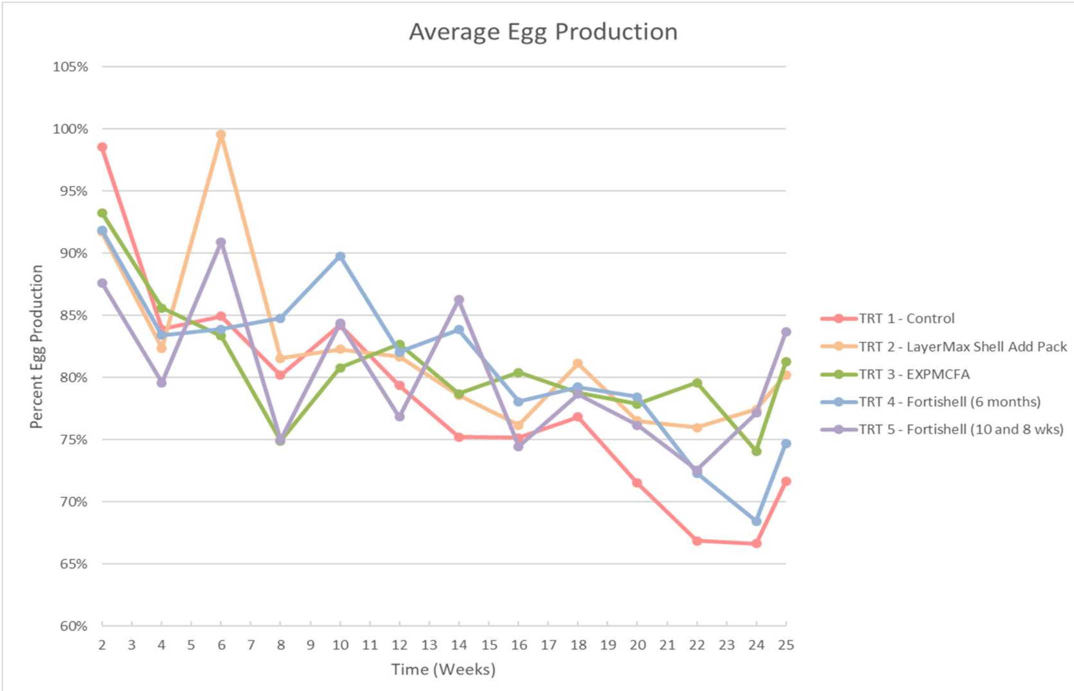


Figure 2.2. Feed Intake
Diet: P<0.0007; Time: P<0.0001; Diet x Time: NS

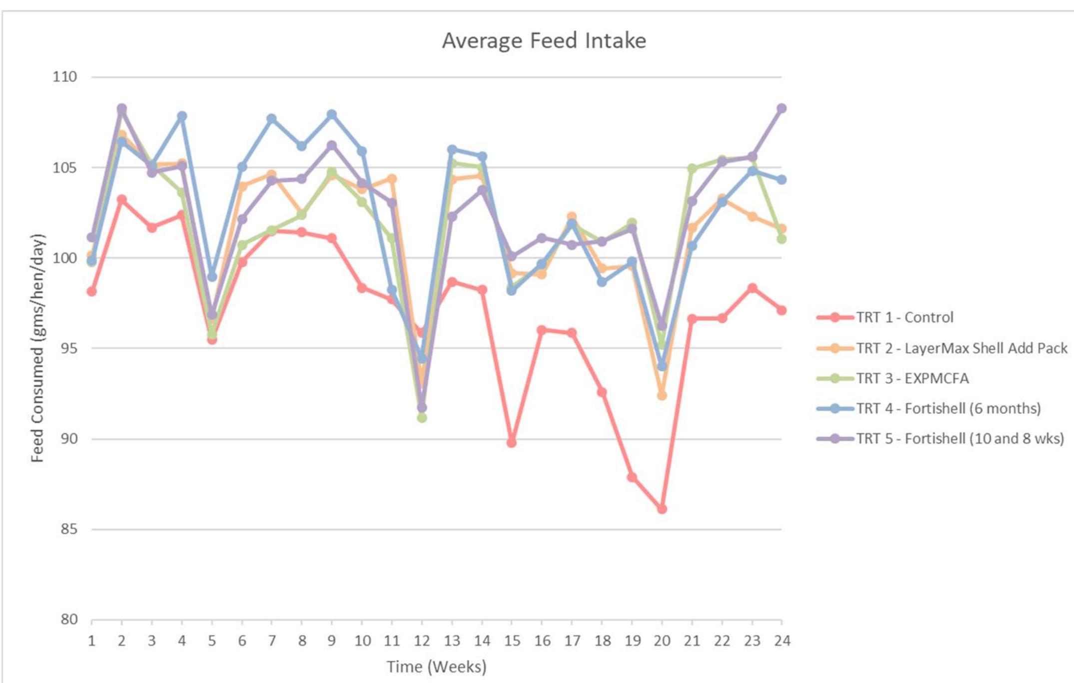


Figure 2.3. Egg Weight
Diet: P<0.0012; Time: P<0.0001; Diet x Time: NS

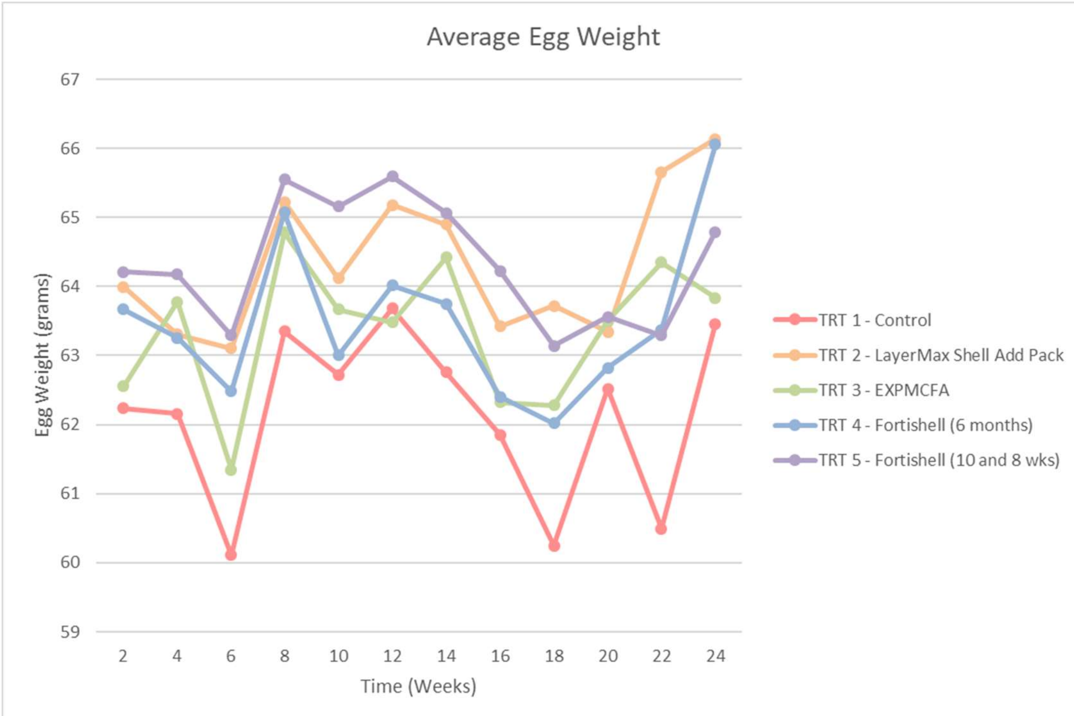


Figure 2.4. Egg Mass
Diet: P<0.140; Time: P<0.001; Diet x Time: P<0.001

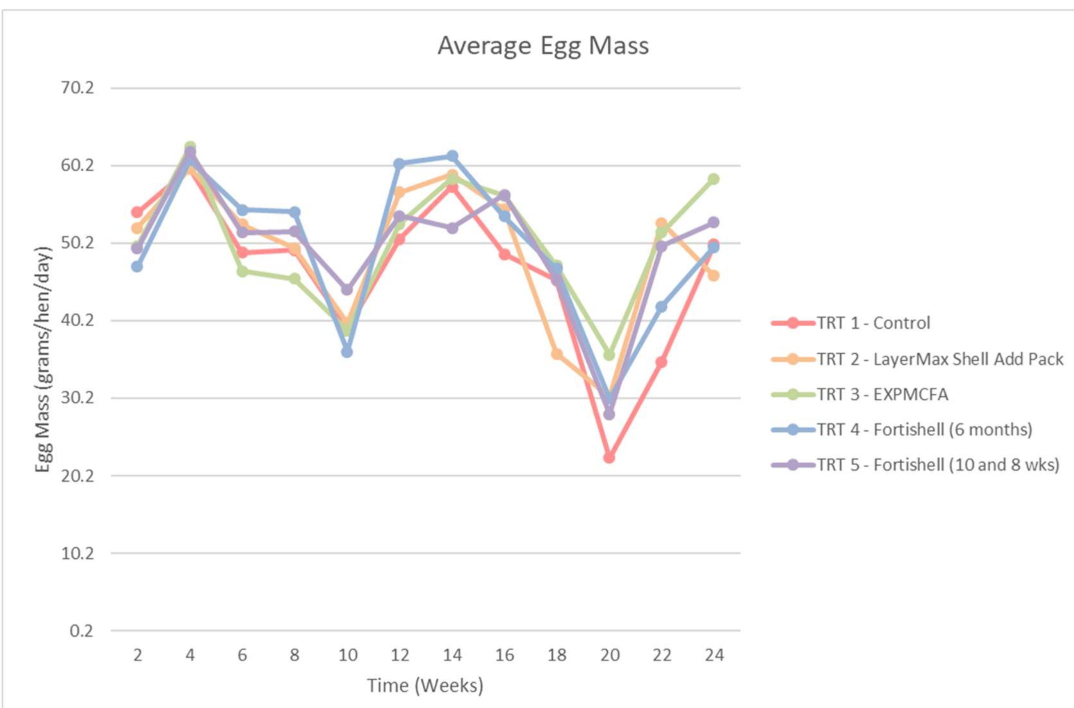


Figure 2.5. Eggshell Percent
Diet: NS; Time: P<0.001; Diet x Time: NS

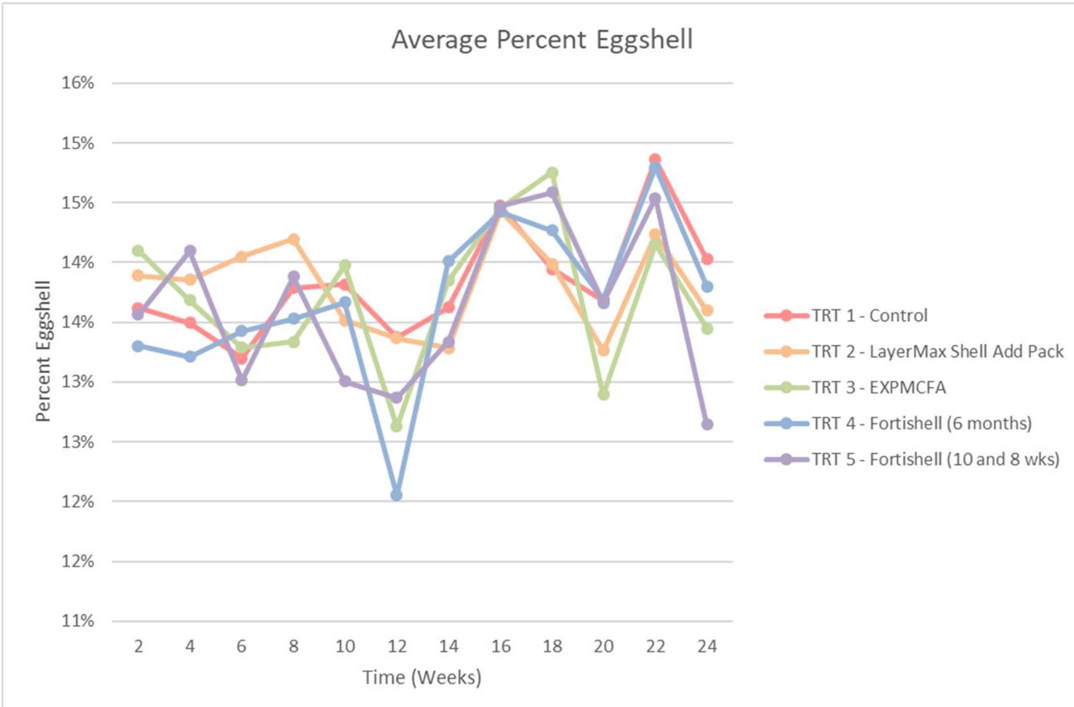


Figure 2.6. Haugh Unit
Diet: P<0.624; Time: P<0.001; Diet x Time: P<0.772

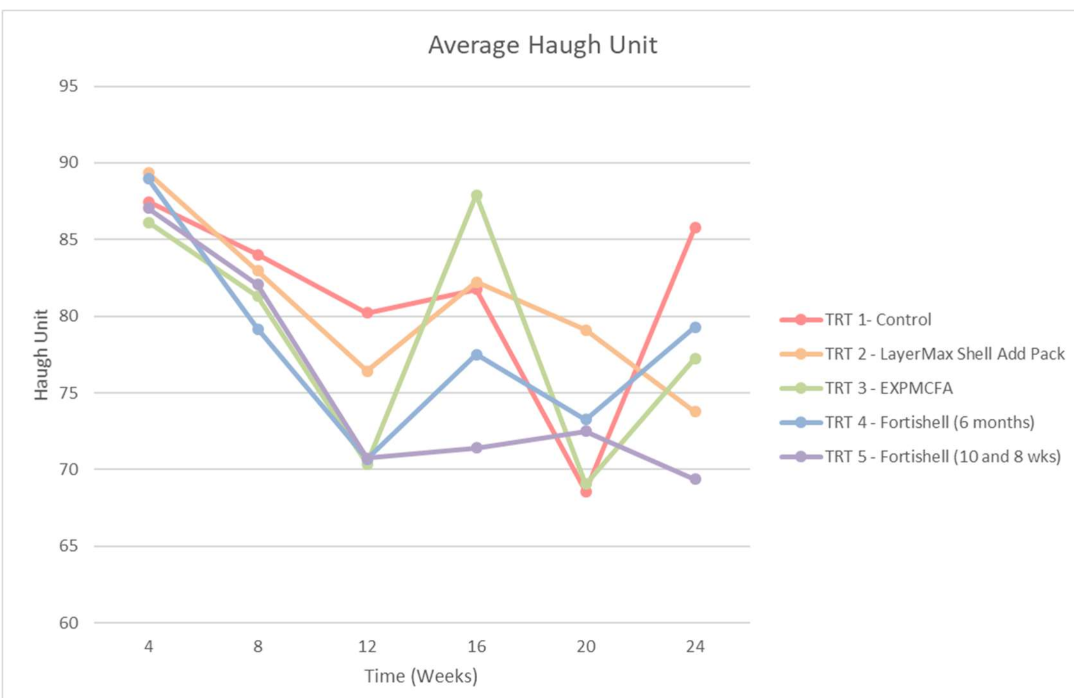
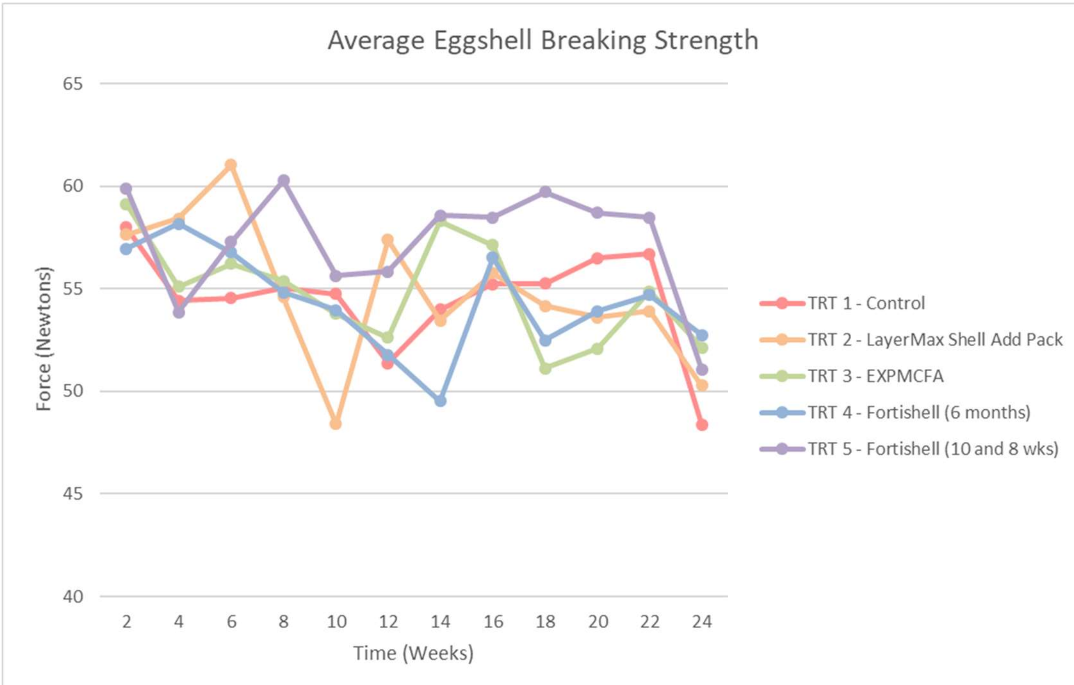


Figure 2.7. Eggshell Breaking Strength
Diet: P<0.12; Time: NS; Diet x Time: NS



The effect of a novel butyric acid product on egg production parameters and nutrient digestibility in 85 week old White Leghorns.

By: S. Purdum[1], J. Foley[1], R. Sygall[2]
[1]University of Nebraska – Lincoln
[2]Perstorp Feed and Food Corporation

ABSTRACT: A novel butyric product – butyric acid bound to glycerol forming a triglyceride was fed to an older laying hen flock from 86 to 100 weeks of age to measure effects egg production parameters and shell quality. Source of butyric acid was ProPhorce SR from Perstorp Corporation. Two treatments were fed to Bovan White Leghorn hens (control or butyric acid (500 g/ton) housed in a traditional cage unit with 12 replicate cages (3 hens/cage) for a 14-week period. Basal diets were corn/soy based diets with 10% DDGS with a total Ca level of 4.6%. Parameters measured included daily egg production, feed intake, biweekly egg weights, eggshell %, eggshell breaking strength (texture analyzer) and Ca and P digestibility marker study utilizing titanium as the marker. Results show no significant effects between treatments for feed intake ($p<0.9027$), egg production ($p<0.4466$), egg weight ($p<0.1346$), egg mass ($p<0.2618$), eggshell percent ($p<0.8470$), instance of shell less eggs ($p<0.2973$), calcium digestibility ($p<0.9740$), or phosphorus digestibility ($p<0.2834$). A significant interaction between diet and time was noted for feed intake ($p<0.0656$), with the butyric acid supplemented group showing higher feed intake in weeks 5-15. There was a trend toward significant in eggshell breaking strength ($p<0.0876$)

Key Words: Layers, Butyrin, Eggshell

Chapter 3: The effect of a novel butyric acid product on egg production parameters and nutrient digestibility in 85 week old White Leghorns.

INTRODUCTION

As the laying hen ages, more challenges arise in the egg production process. The hen lays larger eggs as she ages, but ability to produce eggshell stays about the same. This results in a weaker shell and an increase in broken eggs. Hens also experience weakening intestinal mucosa and shortening villi as they age, resulting in a decrease in overall nutrient absorption. There is a smaller nutrient pool available to the hen, including calcium, which increases a need for calcium from bone turnover to make an eggshell. With the hen using a portion of the calcium stored in her skeletal structure for the eggshell formation process, her skeleton becomes weaker. Osteoporosis becomes a concern. Some hens may even experience cage layer fatigue, an extreme condition brought about by a weakened skeletal structure that leads to a collapse of the spine and paralysis. When one combines the increase in broken eggs with an increase in mortality or morbidity, the economic loss to farmers becomes great. Therefore, it is important to look into ways to enhance eggshell integrity late in the laying cycle.

Many types of feed additives have been tested over the years. Calcium is supplemented in laying hen diets, but simply increasing calcium cannot address weak egg shell concerns as too much calcium in the diet may have an anorexic effect (Pavlovski et al., 2012).

Organic acids have been shown to promote performance in numerous ways. They have been shown to increase nutrient digestibility, stimulate digestive enzymes, modify intestinal microflora load, improve intestinal epithelial integrity, and are known to be a major component of cellular metabolism in all tissues (Guilloteau et al., 2010). Organic acids are also a naturally occurring substance that is widely accepted. Butyric acid specifically is naturally occurring in the hindgut of humans and food animals due to carbohydrate fermentation (Ricke, 2003). It is available as the Na, K, Mg, or Ca salt, which is preferred as these salts are generally odorless and stable. These characteristics maintain the palatability of feed and address manufacturing concerns with volatility.

Multiple studies have shown positive effects of butyric acid supplementation on egg production and shell strength in laying hens. A study in 2006 showed increased egg production in hens supplemented with butyric acid (Yesilbag and Colpan, 2006). Other studies have shown similar results, with improved egg production in hens aged 70 weeks that were supplemented with butyric acid (Soltan, 2008, Rahman et al., 2008). Egg shell strength was also reported to be improved in several studies (Park et al., 2002, Sengor et al., 2007, Soltan, 2008, Rahman et al., 2008, Chou et al., 2014). It has been suggested that this improvement in eggshell strength may be due to increased mineral and amino acid absorption.

Butyric acid comes in multiple forms. Encapsulated butyric acid is a common form used in animal feeds. Fat-coating the acid prevents dissociation in the stomach, allowing the acid to reach distal parts of the intestine (Hu and Guo, 2007). When butyric acid is present earlier in the digestive tract of birds, it may promote an anorexic response (Moquet et al., 2018). Therefore, the small intestine is the target in many poultry studies. Recently, the Perstorp Company of Malmo, Sweden[8] has tested a novel form of butyric acid. The tributyrin ester is a glycerol ester that consists of 3 butyrate molecules attached to a glycerol backbone (see Figure 3.1 in Appendix 3). This mimics the encapsulation effect and allows the molecule to reach the small intestine

[8] Perstorp Holding AB Neptunigatan 1, 211 20 Malmo, Sweden

before being digested. This study focused on the effects of this tributyrin ester on performance and egg parameters in older laying hens.

MATERIALS AND METHODS

Birds and Housing

A flock of 144 Bovan (ISA North America, Ontario, Canada) White Leghorn hens aged 86 weeks were used for this 16-week trial, from 86 to 102 weeks of age. The trial ran from June 2016 to September 2016. Hens were housed in 24 cages in a Big Dutchman manure belt battery[9], with 3 tiers of 8 cages and 6 hens per cage. Cages measured approx. 45.72 cm tall in the front, 40.005 cm tall in the back, 60.96 cm wide, and 51.435 cm deep, providing hens with approx. 205.74 cms^2 / hen. Water was available ad libitum via a nipple drinker at the back of the cage and hens were provided access to 110 grams/day of feed. The photoperiod consisted of 16 hours of light and 8 hours of dark per day, provided by an automated lighting system. Each cage was randomly assigned to 1 of 2 treatment diets with 12 replicates per treatment using a completely randomized design. The conditions of the trial were approved by the Institutional Animal Care and Use Committee at the University of Nebraska – Lincoln and the trial was held in Poultry Building F on East Campus at the University of Nebraska – Lincoln, Department of Animal Science.

Diets

The independent variable of this trial was diet and consisted of two treatments. Treatment 1, the control diet, consisted of a typical corn-soybean basal diet that follows the NRC

[9] Big Dutchman USA 3900 John F. Donnelly Dr. Holland, MI 49424

recommendations of 1994. The complete control diet composition can be seen in Table 3.1 of Appendix 3. Treatment 2, the experimental diet, consisted of the control feed with a butyric acid supplement added at a 0.055% inclusion rate (approx. 500 grams per ton). The butyric acid was the powder form of tributyrin ester manufactured as ProPhorce by the Perstorp Company (Neptunigatan 1, 211 20 Malmo, Sweden).

Measurements

Egg production, feed consumption and mortality were recorded daily. Egg weight, shell breaking strength, shell percent, and calculated feed conversion was measured biweekly. Calcium and phosphorus digestibility was measured at the trials end.

Egg production was recorded on a daily basis and average weekly egg production was calculated by dividing the number of eggs collected by the number of hen production days. One day of egg production was collected once every other week to measure egg weight, shell breaking strength and shell percent. Egg weight was measured by placing the whole egg on a tared scale. The egg was then cracked and the eggshell was weighed after all components were removed. Egg mass was calculated by multiplying percent egg production times egg weight for a given replicate pen. Eggshell breaking strength was analyzed using a texture analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY). The force in Newtons necessary to crack the eggshell was graphed using an exponent software (Stable Micro Systems LTD., Surrey, UK).

Feed intake was calculated by taking the total amount of feed weighed out for a set period minus the amount weighed back at the end of a set period. For this trial, the set period was 7 days. Feed intake was calculated as average intake per hen per day by dividing the calculated consumption by the number of hens in each cage and the total number of days in the predetermined time period. Feed conversion was calculated by dividing feed intake by egg mass. Mortality was monitored daily. Total number of mortalities were added per treatment at the end of the trial.

Calcium and phosphorus digestibility was measured at the end of the 14-week trial (hens approx. 100 weeks of age). Titanium dioxide was added to the feed at a rate of 0.4% as an inert marker and fed to the hens for 3 days. On the third day, manure samples and feed samples were taken and placed in individually labeled aluminum pans. Samples were dried in an oven at 100 C for 3 days before being ground with an electric grinder and sifted to remove feathers. A portion of the feed and ground fecal samples were sent to Midwest Labs (Midwest Laboratories, Omaha, NE) for calcium and phosphorus content analysis.

Foil tins were dried in an oven overnight and the initial weight of the tins after drying were recorded. 0.5 g of each feed or fecal sample were weighed into individual foil tins in duplicate and dried in a drying oven overnight. The final weight of the dried tin with sample were recorded. Tin weight was subtracted from the final weight to produce the dry sample weight. Dry matter was calculated using the following equation: dry matter = (dry sample weight / wet sample weight) x 100.

For titanium analysis, 0.5 g of each feed and 0.3 g of all fecal samples were weighed into individually labeled 16.125 Pyrex screw cap test tubes in duplicate. Each tube was place into a metal rack and placed in a furnace at 580 C for 10 hours to ash the samples. Tubes were allowed to return to room temperature before adding 0.8 g Na_2SO_4 to the sample. Then 5.0 ml of concentrated H_2SO_4 was added to the tube, the cap was firmly closed, and the tubes were gently vortexed to mix the solutions and the sample. The tubes were placed in a heating block and the block was set to 120 C. Tubes were vortexed every 24 hours for a total of 72 hours in the heating block. The block was turned off and the tubes were allowed to return to room temperature before each solution was transferred to a 50ml volumetric flash that contained 10ml of nanopure water. The solution was then inverted to adequately mix and allowed to return to room temperature. Finally, each sample was poured into an individually labeled 50 ml Falcon tube and left to sit overnight.

Then, 96-well plates were used for the analysis. The first two columns were reserved for 300ul of standards in duplicate. Then, 300ul was used per feed sample and 100ul was used in combination with 200ul of 1.8M sulfuric acid for fecal samples. The plates were then placed on a shaker for 30 minutes and then placed in a microplate reader (Fluostar Optima, BMG Labtech Inc., Cary, NC) to measure absorption. The program was set to read at 410nm with a 5 level standard using the appropriate analysis software (MARS 3.01R2, BMG Labtech Inc., Cary, NC). A TiO_2 template within the software was used to calculate values, and TiO2 values were corrected for dry matter with the equation (TiO2 x %DM)/100. Digestibility was calculated as follows:

$$\% \text{ Ca digestibility} = 1 - [(\% \text{ dietary Ti}/\% \text{ excreta Ti}) \times (\text{excreta Ca}/ \text{ dietary Ca})]$$

$$\% \text{ P digestibility} = 1 - [(\% \text{ dietary Ti}/\% \text{ excreta Ti}) \times (\text{excreta P}/\text{dietary P})]$$

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS, version 9.4 (SAS Institute Inc., Cary, NC, 2015). All response variables were analyzed using a repeated measures model including the fixed effects of time, treatment and their interaction.

RESULTS AND DISCUSSION

This study looked at comparing a basal laying hen diet to a diet that included a butyric supplement. The hypothesis was that the butyric acid would improve overall performance and production parameters, specifically looking toward egg shell quality.

Overall, hens fed the butyric acid showed no statistically significant differences between the control for any of the parameters as seen in Table 3.2. Mortality was unaffected by treatment ($p>0.05$), with a total mortality count of 8 in Trt 1 and 9 in Trt 2.

Feed intake showed a relevant interaction effect of time x diet ($p<0.0656$), with a 2 gram/day increase overall in hens fed butyric acid as compared to the control group during the later weeks of the trial (Figure 3.2), weeks 5-15. This could result in improved nutrient intake due to improved gut health as reported by a 2007 study (Sengor et al., 2007).

While egg production of hens fed butyric acid was approximately 7% higher overall as compared to hens fed the control diet, it was not statistically significant ($p<0.2857$) (Figure 3.3). This response shows insignificant support of multiple studies that supported increased egg production of 5%-10% in hens fed butyric acid (Soltan, 2008, Rahman et al., 2008).

The hens fed butyric acid showed slightly increased egg weights of 1 gram on average when compared to the control, but this was not statistically significant ($p<0.1346$) (Figure 3.4). Trt 2 noted increased egg weights in weeks 6-14. Total egg mass was, therefore, slightly increased ($p<0.3365$) when birds were fed a diet that included the butyric acid supplement due to a slight, non-significant increase in egg production and egg weight (Figure 3.5). This parameter improvement contradicts other studies that found no change in egg weight or egg mass when using a butyric acid salt (Soltan, 2008, Rahman et al., 2008).

Eggshell breaking strength was 2.6 Newtons higher overall in eggs from hens fed butyric acid ($p<0.0876$), with increased improvement correlated with time the treatment diet was fed, noticeably at 6, 8, 12 and 14 (Figure 3.7). This finding agrees with the study that looked at sodium butyrate supplementation and noted improvements in egg shell strength. (Chou et al., 2014). Improved eggshell breaking strength may be correlated with improved mineral intake and improved nutrient absorption, specifically available calcium to the hen.

There was a trend toward a time x diet interaction effect ($p<0.0907$) on percent eggshell (Figure 3.6), particularly favoring butyric acid at the end of the trial to increase percent eggshell. This is supported by a study of Sobczak and Kozlowski (2016) that found an increase in overall shell percent. Total instance of shell-less eggs was decreased by approximately 26% in the butyric acid group when compared to the control, but this was not statistically significant ($p<0.2973$) (Figure 3.8).

Calcium and phosphorus digestibility were unaffected when hens were fed butyric acid ($p<0.9740$ and $p<0.2834$ respectively) (Figure 3.9). This is consistent with a study done with younger hens that were supplemented with butyric acid that showed no treatment differences in phosphorus digestibility (Hanna et al., 2019). Several studies have shown that butyric acid may improve overall efficiency of the gastrointestinal tract and nutrient digestibility (Guilloteau et al., 2010, Park et al., 2009, Khan and Iqbal, 2015).

CONCLUSION

Deteriorating gut health and egg shell strength in older laying hens has been a perplexing challenge to researchers and nutritionists for years. Butyric acid in the form of tributyrin showed some indications of improved percent eggshell and eggshell breaking strength during this trial. The literature discussed showed that variability in parameter effects are expected as researchers are still working to determine the right supplementation of butyric acid in hens. Continued research into the effects of butyric acid is very important as researchers continue to work to decrease egg waste due to broken shells, thereby improving economic performance for producers.

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Table 3.1. Composition of Treatment Diets

Ingredient (%)	Control	Butyric Acid
Ground Corn	54.20	54.20
Soybean Meal	19.61	19.61
DDGS	10.00	10.00
Limestone	11.28	11.28
Vegetable Oil	3.14	3.14
Dicalcium Phosphate	1.00	1.00
Salt	0.32	0.32
Vitamin/Trace Mineral Premix*	0.20	0.20
Methionine	0.12	0.12
Lysine	0.04	0.04
Butyric Acid* (Tributyrin)	0.00	0.055

Nutrient Analysis		
ME, Kcal/kg	2800	2800
Protein, %	16.2	16.2
Lysine, %	0.80	0.80
Methionine, %	0.39	0.39
TSAA, %	0.70	0.70
Calcium, %	4.6	4.6
Available P, %	0.35	0.35

*Note: Vitamin/Trace Mineral Premix contained Phytase activity.
*ProPhorce Butyric Acid Source: Perstorp Holding AB Neptunigatan 1, 211 20 Malmo, Sweden.

Table 3.2. Results Summary

Parameter	Control	Butyric Acid	Diet	Time	Diet x Time	SEM
Feed Intake, g/hen/day	102.2	104.88	0.9027	0.0975	0.0656	1.1509
Egg Production, % hen day	60.02	66.68	0.4466	0.0001	0.2294	0.0397
Egg wt, grams	65.93	66.92	0.1346	0.0147	0.1111	0.1093
Egg mass, g/hen/day	40.11	45.08	0.2618	0.0001	0.3365	0.6721
Egg Shell, %	13.46	13.40	0.8470	0.0001	0.0907	0.0014
Egg Shell Breaking Strength, N	39.95	42.67	0.0876	0.0445	0.1982	1.0453
Instance of Shell-less eggs	0.36	0.10	0.2973	N/A	N/A	0.6865
Calcium Digestibility, %	25.91	25.75	0.9740	N/A	N/A	3.2711
Phosphorus Digestibility, %	31.94	28.43	0.2834	N/A	N/A	2.2589

Figure 3.1. 3D Tributyrin Ester Molecule[10]

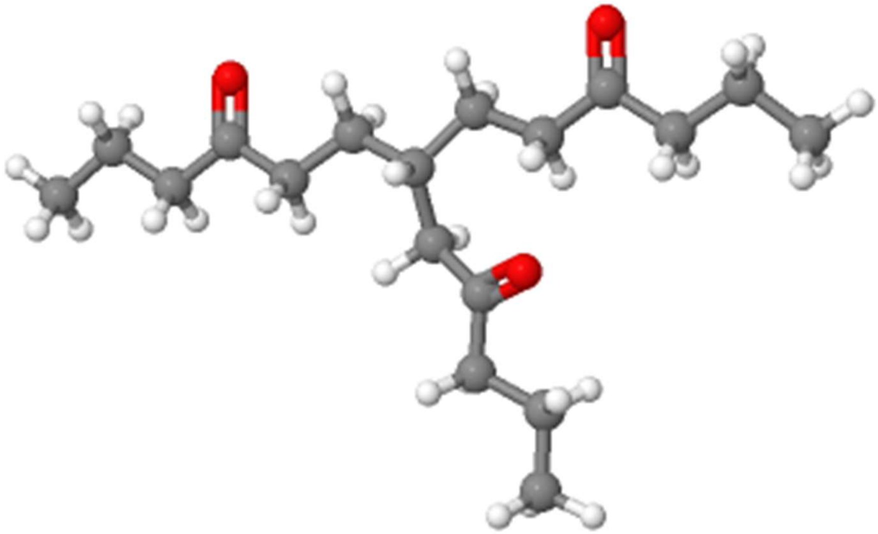
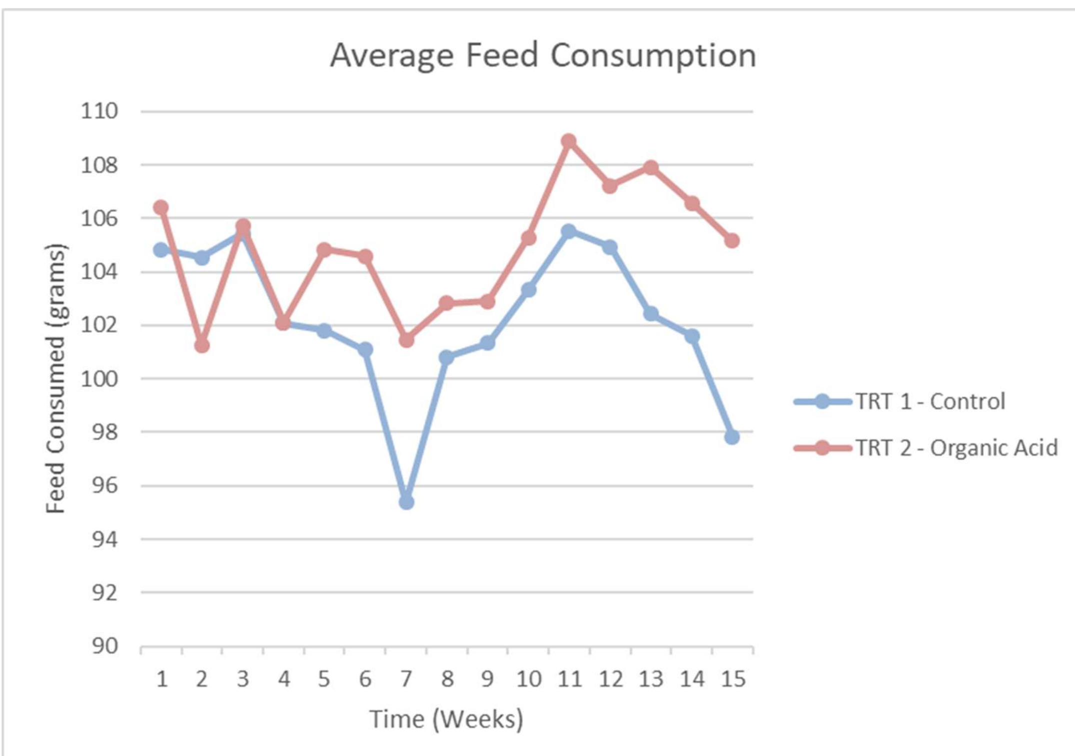


Figure 3.2. Feed Intake
Diet: P<0.9027; Time: P<0.0975; Diet*Time: P<0.0656



[10] Created manually using a molecule simulator program at http://biomodel.uah.es/en/DIY/JSME/draw.en.htm.

Figure 3.3. Egg Production
Diet: P<0.2857; Time: P<0.0006; Diet*Time: P<0.3945

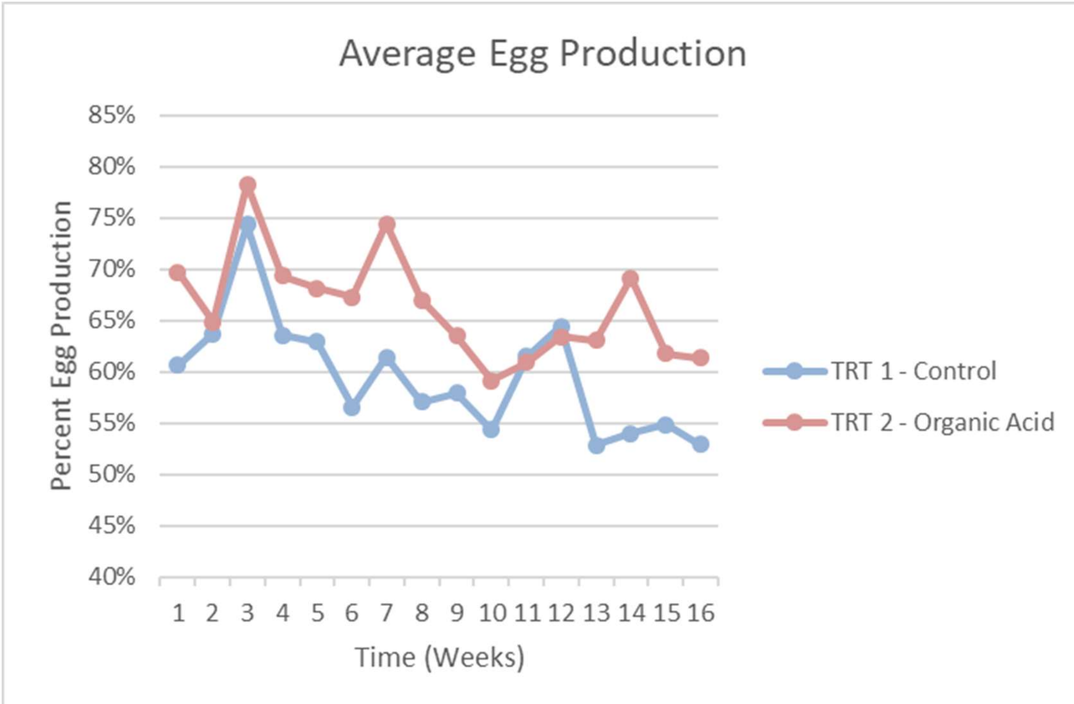


Figure 3.4. Egg Weight
Diet: P<0.1346; Time: P<0.0147; Diet*Time: P<0.1111

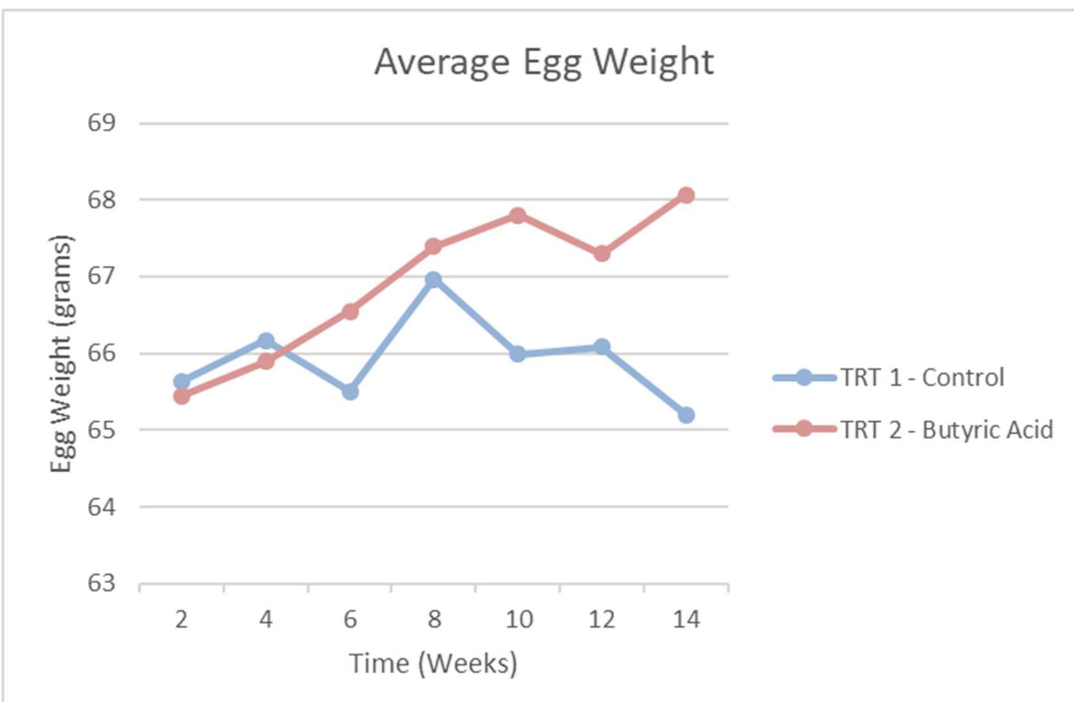


Figure 3.5. Egg Mass
Diet: P<0.2618; Time: P<0.0001; Diet*Time: P<0.3365

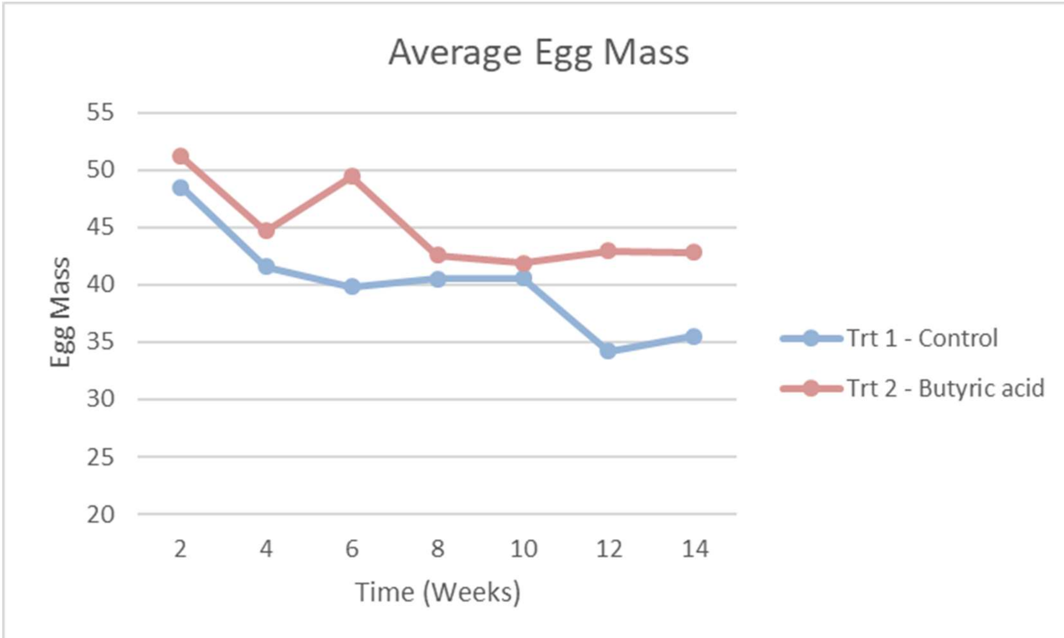


Figure 3.6. Egg Shell Percent
Diet: P<0.8470; Time: P<0.0001; Diet*Time: P<0.0907

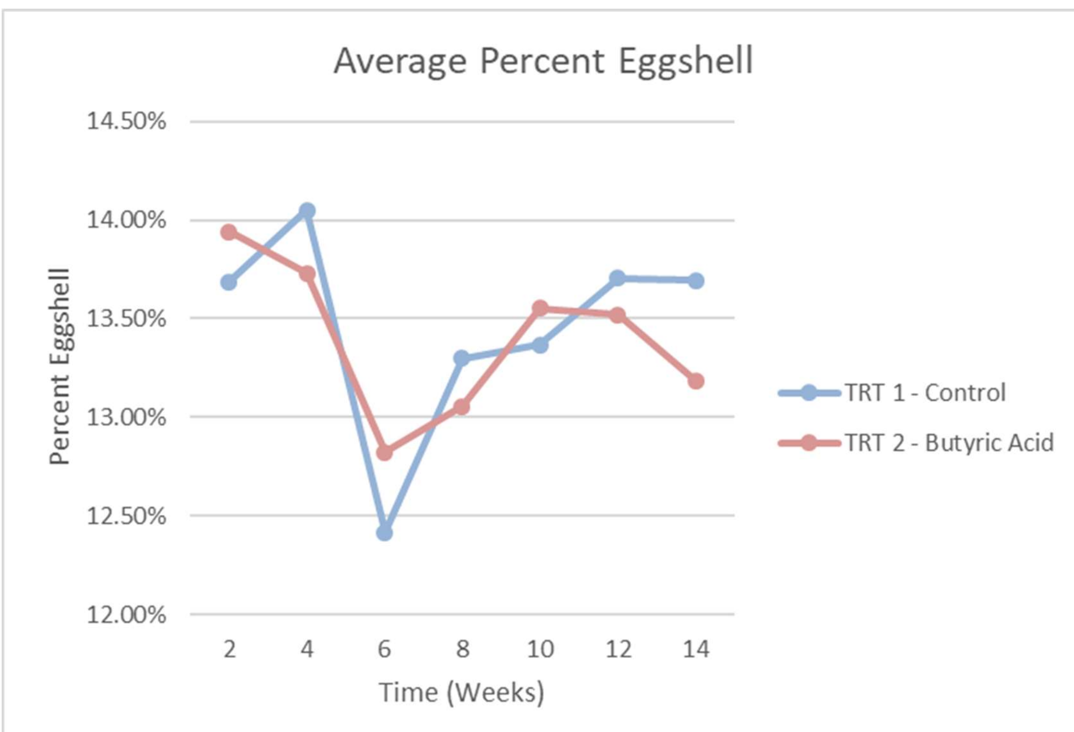


Figure 3.7. Egg Shell Breaking Strength
Diet: P<0.0876; Time: P<0.0445; Time*Diet: P<0.1982

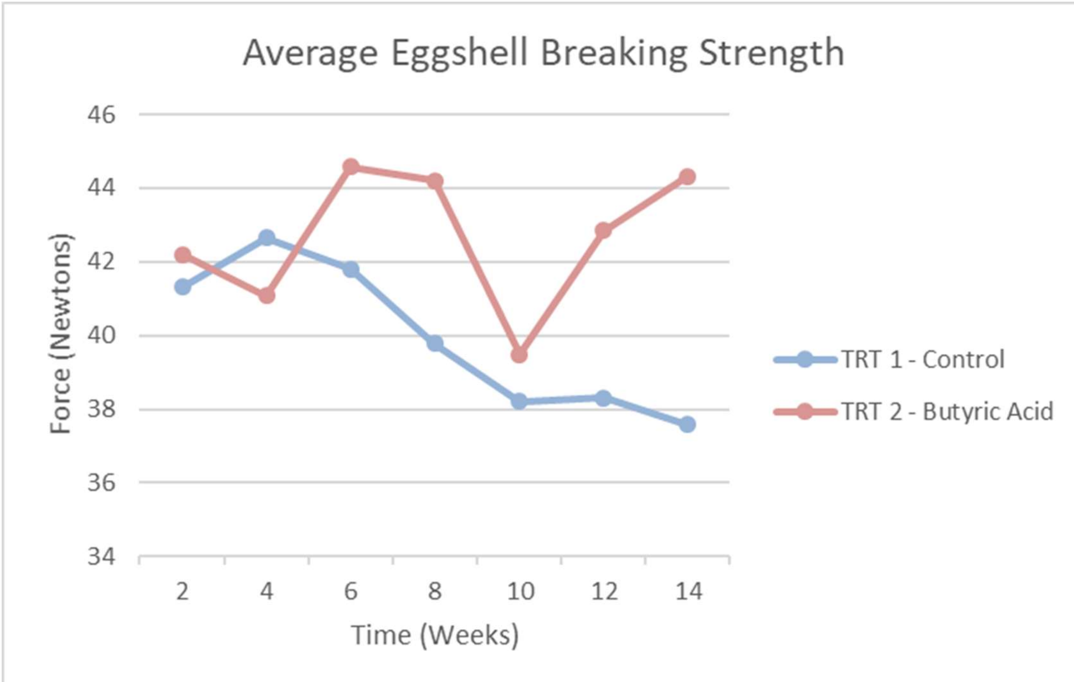


Figure 3.8. Instance of Shell-less Eggs
Diet: P<0.2973

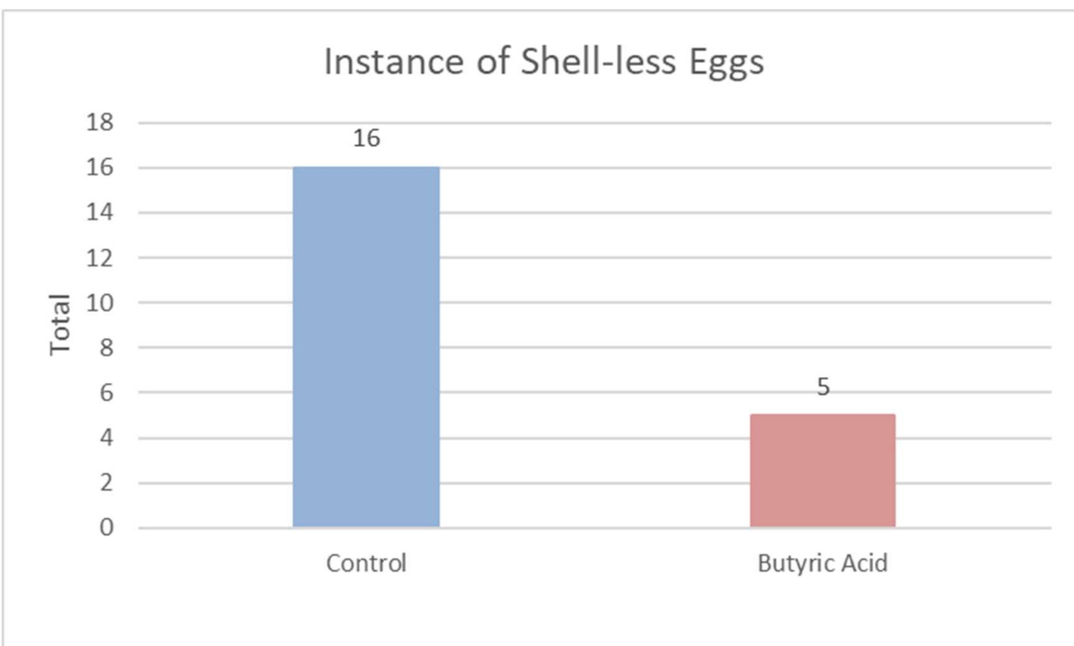


Figure 3.9. Calcium and Phosphorus Digestibility
Calcium Trt: P<0.9740
Phosphorus Trt: P<0.2834